

In Vivo and In Vitro Models for Assessing Drug Absorption Across the Buccal Mucosa

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Abstract The buccal mucosa serves as an attractive site for the systemic delivery of many drugs, including peptides and proteins, because of its inherent permeability and the avoidance of intestinal and hepatic first-pass effects. In order to assess the potential of the buccal mucosa to act as a site of drug absorption in preclinical drug discovery and development, various models have been used. This chapter focuses on the in vivo and in vitro methods that are available to assess buccal mucosal drug absorption, with an emphasis on the advantages and limitations associated with each of the models available and the issues associated with choice of animal tissue used in preclinical drug absorption studies. Most of our work in this field has been performed using porcine buccal mucosa in modified Ussing chambers, and the reader is referred to the appendix (available online) for a comprehensive protocol on this technique.

Keywords: Buccal mucosa; Permeability barrier; Passive diffusion; Excised tissue; Nonkeratinized epithelium; Diffusion chamber

4.1. Introduction

Most drugs are delivered to the systemic circulation by the conventional oral route; however, the systemic absorption of certain drugs can be significantly hindered by the environment of the gastrointestinal tract. Drugs that are susceptible to acid hydrolysis, are substrates for the various efflux mechanisms present in the intestinal wall, or have significant intestinal or hepatic metabolism may exhibit poor bioavailability when administered via the oral route [8]. With the advent of the biotechnology era, drug candidates with such properties are emerging, and so alternative routes of drug administration are becoming more necessary.

The buccal mucosa, which lines the inside of the cheek, has been investigated as an alternative route for drug delivery, especially for proteins and peptides. There are many advantages associated with the use of the buccal mucosa as a site for the delivery of drugs into the systemic circulation. Since blood flow from the buccal epithelium drains directly into the internal jugular

vein [66, 75], first-pass metabolism in the liver and intestine can be avoided. Additionally, the mucosa lining the oral cavity is easily accessible, which ensures that a discrete dosage form can be applied to the required site and can be removed easily in the case of an emergency [27, 96, 103]. However, like the skin, the buccal mucosa acts as a barrier to the absorption of xenobiotics, and may hinder the absorption of drugs into the systemic circulation. Consequently, preclinical permeation studies can be used to elucidate whether a compound can be successfully delivered systemically via the buccal route.

4.2. Structure and Environment of the Buccal Mucosa

4.2.1. Epithelial Organization

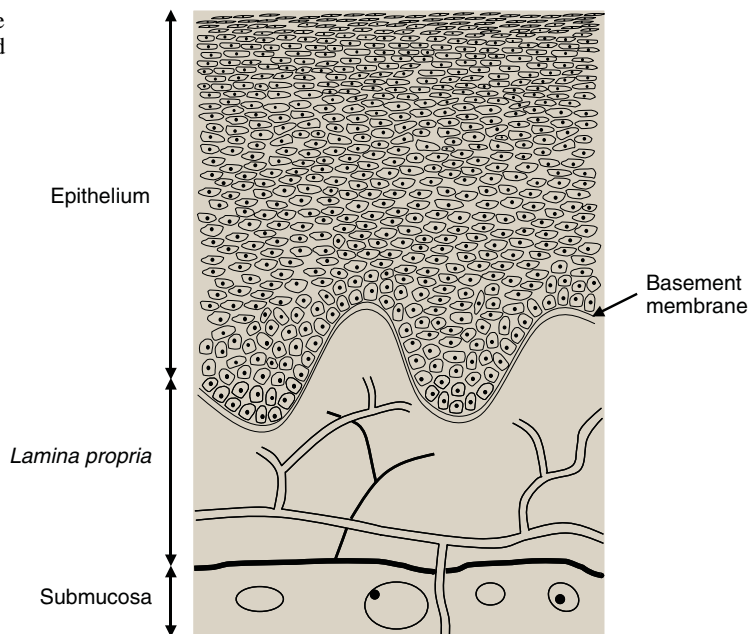
Given that the primary role of the buccal mucosa, like the skin, is to protect underlying tissues and organs from foreign agents, it is not surprising that this tissue is highly structured. The surface of the buccal mucosa consists of a stratified squamous epithelium which is separated from the underlying connective tissue (*lamina propria* and submucosa) by an undulating basement membrane (a continuous layer of extracellular material $\sim 1\text{--}2\ \mu\text{m}$ in thickness) [95]. This stratified squamous epithelium consists of differentiating layers of cells (keratinocytes), whose size, shape, and content change as the cells travel from the basal region to the superficial region [21]. The superficial cells of the nonkeratinized buccal mucosa, unlike corneocytes of the *stratum corneum*, retain their nuclei and some cytoplasmic function, and are surrounded by a cross-linked protein envelope [125]. There are $\sim 40\text{--}50$ cell layers in the buccal mucosa, resulting in a tissue with a thickness of $500\text{--}600\ \mu\text{m}$ [37, 42, 100]. While the epithelium serves as a mechanical barrier, the *lamina propria* provides mechanical support and carries the blood vessels and nerves, which supply nutrition and innervation to the epithelium, respectively. The general structure of the buccal mucosa is shown in Figure 4.1.

4.2.2. Organization of the Intercellular Domain

As cells migrate to the upper third of the epithelium, membrane-coating granules become evident at the superficial aspect of the cells. While the appearance of membrane-coating granules in the epidermis has been well characterized [43, 74, 90,], less is known about their nature in nonkeratinized epithelia, albeit their existence has been demonstrated [120, 121]. The membrane-coating granules are small cytoplasmic granules, $\sim 2\ \mu\text{m}$ in diameter, which appear in the Golgi region of the prickle cell layer, migrate to the superficial region of cells at the midlevel of the epithelium, and apparently fuse with the cell membrane in the upper third-quarter of the epithelium [120]. It is upon fusion with the cell membrane that the contents of the membrane-coating granules are extruded into the intercellular spaces of the epithelium [68].

The membrane-coating granules in keratinized epithelia contain electron-dense lipid lamellae [68, 77], and therefore, the intercellular spaces of the *stratum corneum* are filled with short stacks of lipid lamellae [67, 132]. Most of the membrane-coating granules in nonkeratinized epithelia consist of amorphous material [120]; however, some studies have shown that a small number of these granules in nonkeratinized epithelia contain lamellae [151]. Therefore,

Figure 4.1 General structure of the buccal mucosa. Taken and modified with permission from Harris et al. [42].



the intercellular spaces of the superficial layer of nonkeratinized epithelia contain electron lucent material, which may represent nonlamellar liquid phase lipid, with occasional short stacks of lipid lamellae [69]. It is this absence of organized lipid lamellae in the intercellular spaces of the buccal mucosa which results in a greater permeability to exogenous compounds, compared with keratinized epithelia [123].

4.3. The Barriers of the Buccal Mucosa

4.3.1. Location of the Permeability Barrier

The upper one-third to one-quarter of the buccal epithelium is generally responsible for the barrier properties of the buccal mucosa. This was originally reported in 1973, when it was demonstrated that horseradish peroxidase could not penetrate the top 1–3 cell layers of the oral mucosa of monkeys, rabbits, and rats, when applied topically to these surfaces [119]. When applied subepithelially, horseradish peroxidase was found in the connective tissue and extended through the intercellular spaces of the epithelium, up to the region which coincided with the appearance of membrane-coating granules [119]. This led to the suggestion that the permeability barrier of the buccal mucosa may be attributed to the materials extruded from the membrane-coating granules. These experiments were repeated using lanthanum salts, to ensure that this region was also the main barrier to the permeation of smaller molecules, and identical results were obtained [122]. Further evidence to suggest that the barrier properties of the buccal mucosa are due to the extruded materials of the membrane-coating granules comes from studies assessing the permeability of tissues lacking such granules. An example of such tissue is the junctional

epithelium which attaches the gingival stratified squamous epithelium to the tooth surface [100]. When horseradish peroxidase and microperoxidase were applied to the epithelial surface of this tissue or injected subepithelially, both proteins penetrated through the intercellular spaces of the entire epithelium [97, 133], giving evidence that the lipids extruded by these membrane-coating granules are the major hindrance to permeability across the buccal mucosa.

4.3.2. Chemical Nature of the Permeability Barrier

It is generally accepted that the barrier of the nonkeratinized oral epithelium is composed of lipid material, since treatment of oral mucosa with chloroform/methanol mixtures has resulted in a reduced barrier function [126]. To verify the chemical nature of these lipids, various regions of porcine oral cavity have been separated and the lipids present in each region have been extracted and identified by thin-layer chromatography [69, 126, 127, 129, 152,]. Similar to porcine epidermis, the keratinized palatal and gingival mucosae contained high quantities of ceramides and cholesterol, and a low proportion of cholesterol esters and glycosylceramides. In contrast, the buccal and sublingual mucosae, both of which are nonkeratinized, contained higher quantities of the more polar phospholipids, cholesterol esters, and glycosylceramides, and minimal amounts of ceramides. Histochemical staining suggested that the polar lipids were localized in the intercellular spaces of the nonkeratinized oral epithelium [129]. Therefore, the intercellular lipids of the nonkeratinized regions of the oral cavity are of a more polar nature than the lipids of the epidermis, palate, and gingiva, and this difference in the chemical nature of the lipids may in part contribute to the differences in permeability observed between these tissues [126].

4.3.3. Other Permeability Barriers in the Buccal Mucosa

In addition to the intercellular lipids of the buccal mucosa, there appear to be other barriers which may reduce the ability of an exogenous compound to permeate the buccal mucosa. These include the salivary film and mucus layer, the basement membrane, and a metabolic barrier.

4.3.3.1. Salivary Film and Mucus Layer

Unlike the skin, the mucosae of the oral cavity are constantly maintained moist by the continual secretion of saliva from the three major salivary glands (submandibular, parotid, and sublingual) and the minor salivary glands located in or beneath the mucosae. The thickness of the salivary film (pellicle) has been calculated to be between 70 μm and 100 μm [23]; however, both the composition and amount of saliva produced can vary between individuals, with time of day, disease states, and drug therapy [33], all of which must be taken into account when considering the oral cavity as a site for drug delivery.

The function of the mucosal pellicle is to serve as a barrier between the oral epithelial surface and the external environment, and so it may also act as a barrier to drug delivery. However, there are limited studies assessing the role of the mucus layer in buccal permeability. In one study, treatment of the oral mucosa with anticholinergic agents resulted in an increased permeability of certain compounds, and it was suggested that the reduced salivary flow may have been responsible for the reduced barrier properties of the tissue [113]. In

another study, addition of human submandibular saliva to the surface of rabbit oral mucosa reduced the initial rate of transport of tritiated water; however this effect was transitory [1]. Therefore, the role of mucus in the overall permeability barrier of the buccal mucosa is not clear, and any hindrance provided by the mucus layer may be negligible relative to the inherent barrier nature of the epithelium. The presence of mucins in the mucosal pellicle may also be exploited by the formulation scientist for mucoadhesive purposes, and this advantage may outweigh any slight improvement in permeability achieved by removing or manipulating this layer.

4.3.3.2. Basement Membrane

While the superficial layers of the buccal mucosa represent the primary barrier to exogenous compounds, there is evidence to suggest that the basement membrane may be important in limiting the transport of some agents from the epithelium into the underlying connective tissue. The basement membrane may act as a barrier to the transport of large molecules, including immune complexes [135] and proteins [4]; however, there is no clear relationship between the size of permeant and the hindrance provided by the basement membrane. This was shown in a study where the basement membrane acted as a major barrier to inulin (5,000 Da) and dextran 70 (70,000 Da) but not to dextran 20 (20,000 Da) [5]. Additionally, the basement membrane has been shown not to limit the transport of the large protein, horseradish peroxidase [119], but restricts the absorption of smaller β -adrenoceptor-blocking agents and the nucleoside reverse transcriptase inhibitor, 2', 3'-dideoxycytidine [28, 153]. This suggests that the charge on molecules may be important in nonspecific binding to components of the basal lamina [125], rather than the basal lamina having a simple sieving function. Although the basement membrane may provide some resistance to the permeability of some compounds, a greater body of evidence suggests that the superficial layers of the buccal epithelium are largely responsible for the barrier properties of the buccal mucosa [119, 122–124].

4.3.3.3. Metabolic Barrier

Although the buccal mucosa is an alternative route for compounds which undergo extensive hepatic metabolism, there is some potential for biotransformation in the oral cavity, particularly for peptides and proteins. Proteolytic activity has been identified in buccal tissue homogenates from various species and a number of peptides have been shown to undergo degradation in such homogenates [56, 86, 156]. However, as the location of the peptidase (intracellular or extracellular) cannot be determined from these experiments, it is difficult to assess whether these enzymes will come into contact with proteins and affect their subsequent penetration across the mucosa. To assess this, the permeability of insulin in the presence of an enzyme inhibitor was investigated. While the degradation of insulin in rabbit buccal homogenate was strongly inhibited by the serine protease inhibitor aprotinin [156], the enzyme inhibitor had no effect on the buccal absorption of insulin in vivo [9]. Because of the large molecular weight of insulin, it is unlikely to enter the epithelial cells of the mucosa as it traverses the tissue, and since the transport of insulin was not affected by aprotinin, this suggested that the protease was present intracellularly. It is therefore unlikely that these proteases will act as a significant barrier

to compounds which do not enter epithelial cells as they permeate the buccal mucosa.

A recent study, however, has shown that aminopeptidase activity is present on the surface of porcine buccal mucosa, and that various aminopeptidase inhibitors, including amastatin and sodium deoxycholate, reduce the mucosal surface degradation of the aminopeptidase substrate, leucine-enkephalin [149]. Since the peptidases are present on the surface of the buccal mucosa, they may act as a significant barrier to the permeability of compounds which are substrates for the enzyme. In addition to proteolytic enzymes, there exist some esterases, oxidases, and reductases originating from buccal epithelial cells, as well as phosphatases and carbohydrases present in saliva [154], all of which may potentially be involved in the metabolism of topically applied compounds.

4.4. Mechanisms Involved in Oral Mucosal Absorption

Substances can be transported across epithelial membranes by simple passive diffusion, carrier-mediated diffusion, and active transport, in addition to other specialized mechanisms, including endocytosis.

4.4.1. Passive Diffusion

The major mechanism involved in the transfer of a drug across the oral mucosa is described by simple Fickian or passive diffusion of the un-ionized form of the drug in accordance with the pH-partition hypothesis [75, 117]. This was first demonstrated for buccal absorption with a series of amphetamines [13]. In this study, drug transport appeared to be a passive diffusion process since optical isomers of a drug were absorbed to the same extent; absorption was dependent on the concentration of un-ionized lipid-soluble form of the drug; and no difference in the percentage absorption of the drug at any given pH value was observed when the drug was given separately or as a mixture with other drugs. Since this initial finding, there have been many studies demonstrating the passive nature of transfer across the oral mucosa [6, 14–17, 20, 22, 25, 26, 44, 94, 101, 110, 130]. Under these conditions of passive diffusion, the physicochemical properties of the membrane and the drug dictate the transport rate across the biological membrane [96].

4.4.2. Carrier-Mediated Transport

Although passive diffusion is the major transport mechanism for drug permeation across the buccal mucosa, the absorption of certain nutrients from the oral cavity has been shown to involve carrier systems. The absorption of D-glucose and L-arabinose across the buccal mucosa was shown to be both saturable and stereospecific [73]. This indicated the presence of a carrier-mediated transport system for these sugars, since saturation and stereospecificity are not characteristics of a passive diffusion process. Additionally, the absorption of D-glucose, galactose, and 3-*O*-methyl-D-glucose was at least partly dependent on the presence of sodium ions, and the transport of D-glucose was inhibited by galactose and 3-*O*-methyl-D-glucose, suggesting that there was at least one common carrier system in the buccal mucosa [73]. Such a specialized

mechanism for D-glucose transport was also observed in a cultured stratified cell layer of human oral mucosal cells [57]. In another study assessing the absorption of D-glucose at various sites in the oral cavity, absorption was found to be saturable only in the dorsum of the tongue, and these authors suggested that a specialized transport system for D-glucose existed only at this site [60]. However, using Western blot analysis, various glucose transporters have been identified in cells of the buccal mucosa as well as the dorsum of the tongue and the buccal mucosa [92]. Therefore, greater clarification is required in this area.

In addition to sugars, the absorption of various vitamins, including L-ascorbic acid, nicotinic acid, and nicotinamide, have been shown to be dependent on the presence of sodium ions, indicating absorption from the oral cavity by carrier-mediated processes [35, 99]. When the absorption of thiamine was investigated *in vivo*, absorption rates showed saturation at high concentrations of the vitamin [34], giving further support to the finding that carrier-mediated processes are involved in the oral mucosal absorption of some nutrients. Recent investigations have also indicated the existence of an energy-dependent carrier-mediated monocarboxylic acid transporter system in primary cultures of rabbit and hamster oral mucosal cells, and in hamsters *in vivo* [141, 140]. Such carrier-mediated systems may be important in the transport of certain drugs, such as salicylic acid. It has also been shown that the absorption of cefadroxil, an aminoccephalosporin antibiotic, is absorbed in the human oral cavity via a specialized transport mechanism, since its absorption demonstrated saturation phenomena and was inhibited in the presence of another aminoccephalosporin, cephalexin [65]. Therefore, evidence is building to suggest that passive diffusion of compounds may not be the only mechanism by which compounds permeate the buccal mucosa.

There has also been a report regarding the active transport of antibacterial agents in oral mucosa. In a cell line derived from oral epithelium, the uptake of ciprofloxacin and minocycline was not only saturable and inhibited in the presence of other compounds, but the intracellular levels of both antibiotics were 8–40-fold higher than the extracellular levels as well, demonstrating an active transport process [18]. Whether the permeability of these compounds across the entire oral mucosa occurs via an active transport process, however, remains to be determined.

4.5. Methods Employed to Assess the Permeability of the Buccal Mucosa

There have been a range of models used in the preclinical setting to assess the permeability of compounds across the buccal mucosa. While *in vivo* methods are often more appropriate in terms of assessing bioavailability via this route, *in vitro* and *in situ* methods have been instrumental for preclinical compound screening, elucidating mechanisms of transport across the buccal mucosa, and assessing the potential of chemical penetration enhancers for improvement of buccal transport. While this chapter mainly focuses on the *in vivo* and *in vitro* models available for assessing drug permeability across the buccal mucosa, novel cell culture techniques will also be briefly discussed.

4.5.1. In Vivo Methods

4.5.1.1. Buccal Absorption Test

One of the most common in vivo methods used to assess the permeability of the buccal mucosa is the buccal absorption test of Beckett and Triggs [13]. In this test, a known volume of a drug solution is introduced into the oral cavity of a subject, who swirls it around for a specified period of time and then expels it. The subject then rinses his or her mouth with an aliquot of distilled water or buffer solution, and the expelled drug solution and rinse are combined and analyzed for drug content. The difference between the initial and final drug concentration in the solution is assumed to be the amount of drug taken up into the oral mucosa.

The buccal absorption test of Beckett and Triggs has been modified slightly by various investigators. To account for the production of saliva throughout the test, a correction factor was included by Dearden and Tomlinson [29]. Others have added a marker compound into the swirling solution, such as phenol red or polyethylene glycol, to account for salivary dilution and accidental swallowing of the solution [6, 101, 137]. Since kinetic profiles cannot be determined using the original buccal absorption test, Tucker modified the test by taking small samples of the swirled solution from the oral cavity every few minutes without removing the entire test solution [137]. The major benefit of this is that the absorption kinetics of a drug may be studied in a single subject in a simple 15–20-min test.

Although the original and modified buccal absorption tests are easy to perform, do not require blood sampling, and allow for both the rate and the extent of drug loss from the oral cavity to be determined, there are some drawbacks to the method. One of the major disadvantages of this technique is that only the concentration of drug remaining in the oral cavity (swirling solution) is measured, and blood samples are not determined. The amount of drug which disappears from the swirling solution cannot be equated to the amount entering the systemic circulation, due to other factors including membrane storage, potential metabolism, and swallowing of the drug [45, 46]. Since the solution is swirled around the oral cavity, absorption of compound may also occur through all surfaces within the oral cavity, and so the degree to which absorption occurs across a specific site (e.g., buccal and sublingual) remains unknown.

4.5.1.2. Perfusion Cells

To overcome the limitation of nonspecific absorption across all surfaces of the oral cavity and to study regional variation in drug absorption, various absorption or perfusion cells have been designed which can be clamped or attached to particular mucosae within the oral cavity of both animals and humans [12, 59, 60, 91, 93, 94, 155, 158, 159]. In this method, a drug solution is perfused through the cell and the drug absorption is again calculated by drug disappearance from the perfusate. The major drawback with the perfusion cell technique is leakage and large intersubject variation [37]; however, these devices are a major advance in assessing the absorption characteristics of a particular region within the oral cavity, and would be most informative if the appearance of drug in the plasma was simultaneously monitored.

In particular cases where plasma cannot be simultaneously assayed, and there is information available relating the concentration of drug in saliva to the concentration in plasma, it is possible to collect saliva as a surrogate for plasma. Following collection and analysis, an appropriate multiplication factor is incorporated for extrapolation to plasma concentrations. Such a method was recently used to assess the buccal absorption of nicotine in humans [3]. In this study, the disappearance of nicotine from the perfusion solution was used to determine the rate of nicotine absorption, and saliva was simultaneously collected directly from the parotid gland using a modified Carlson-Crittenden cup [11] as a surrogate for plasma. While salivary concentrations increased as perfusion concentrations decreased, it is unknown whether this actually related to an increase in plasma concentrations. Consequently, it is important to determine whether there is a correlation between plasma concentrations and salivary concentrations, if this technique is to be successfully used as a predictor of systemic buccal absorption. However, as informative as such techniques may be, they may not be suitable in the preclinical setting, where high throughput permeability screening is required.

4.5.2. In Vitro Methods

In vitro permeability models are often employed to determine the barrier nature of a particular biological tissue because the diffusion of drugs can be studied in an environment where variables such as temperature, pH, and osmolarity can be easily controlled [157]. When using an in vitro method to predict the absorption of compounds across the human buccal mucosa, an appropriate animal model must be chosen on the basis of its similarity in structure and permeability to the human buccal mucosa. Using the buccal mucosa of an appropriate animal model, in vitro permeability studies are then commonly conducted in diffusion cells. The advantage of in vitro diffusion cells is that the amount of drug that has actually diffused across the tissue can be determined over time, and thus the kinetics of tissue transport may be assessed. There are various diffusion cells that are used in the preclinical screening of compound permeability, including Franz-type diffusion cells, flow-through cells, and modified Ussing chambers.

4.5.2.1. Animal Models

Because of the limited availability of the human buccal mucosa, it is often necessary to use freshly excised mucosa from an alternative animal species. If the role of in vitro studies is to assess the potential of the buccal mucosa as an alternative route for drug delivery in humans, then the buccal mucosa from the animal species chosen should be similar to the human buccal mucosa in terms of permeability, biochemistry, and morphology. While many research groups have used the oral mucosa of rats [10, 115] and hamsters [25, 39, 58, 61–64, 131, 136, 138], these surfaces are keratinized, and so may not be an appropriate model of the nonkeratinized human buccal mucosa. Rabbit buccal mucosa is nonkeratinized and has been used in many in vitro studies assessing the mucosal permeability of compounds [32, 36, 78, 116]; however, the small area of available nonkeratinized tissue often limits its use [125]. The buccal mucosa of dogs and monkeys is nonkeratinized and therefore may be used as a model for the human buccal mucosa; however, the epithelium of the mucosa in these animals is much thinner, and consequently, more permeable than that of

Table 4.1 Epithelial thickness and permeability coefficient (*P*) for tritiated water through the buccal mucosa of different species together with epithelial thickness.

Species	$P \times 10^{-7}$ (cm/min)	Epithelial thickness (μm)
Human	579 $\pm$ 122	580 $\pm$ 90
Pig	634 $\pm$ 60	772 $\pm$ 150
Monkey	1,025 $\pm$ 154	271 $\pm$ 50
Dog	1,045 $\pm$ 37	126 $\pm$ 20

Data are presented as mean $\pm$ SD.
Table taken from Squier et al. 1996 [125]. Copyright (1996) From: Oral Mucosal Drug Delivery, by Michael J. Rathbone (Ed.). Reproduced by permission of Routledge/Taylor & Francis Group, LLC.

humans [2, 76, 87, 114, 125]. The comparative permeability of tritiated water through various animal species and humans is shown in Table 4.1. In addition, the thickness of the buccal epithelium in each species is shown.

Because of the physiologic, anatomic, nutritional, and metabolic similarities between humans and pigs [31], the pig has become a widely used and important animal model for research on human disease. The buccal mucosa of pigs is nonkeratinized and has a similar structure, morphology, and composition to the human buccal mucosa [24, 123, 126]. Additionally, as shown in Table 4.1, the thickness of the epithelia in human and porcine buccal mucosa is fairly similar. While structure and morphology are important determinants in the comparative process, the permeability characteristics of the model tissue must reflect the barrier nature of the human buccal mucosa. The permeability of tritiated water through porcine buccal mucosa has been shown to be very similar to that of the human buccal mucosa [72], and more recently, no significant differences were observed in the permeability of mannitol or testosterone through the porcine and the human buccal mucosa [87]. Because of the large amounts of pig oral mucosa available from slaughterhouses and the similar structure and permeability to human tissue, most laboratories use porcine buccal mucosa when assessing mucosal permeability and the effect of various chemical penetration enhancers on mucosal drug delivery [7, 20, 28, 30, 38, 41, 47, 48, 54, 70, 79, 80–84, 104, 105, 108, 110, 112, 130, 147, 148, 153]. It is therefore recommended that preclinical evaluation of compound permeability across the buccal mucosa be performed with porcine buccal mucosa, as a result of its similar structure and permeability characteristics to that seen in the human buccal mucosa.

4.5.2.2. Franz-Type Diffusion Cells

While Franz-type diffusion cells are commonly used to assess in vitro penetration of compounds across the skin, they have also been used for the assessment of compound permeability across the buccal mucosa [19, 71, 104]. In this system, buccal mucosa is sandwiched between two chambers, and compound solution is added to the donor chamber with compound-free buffer in the receptor chamber. The receptor chamber is then periodically sampled to assess the amount of compound that has permeated the tissue over time.

4.5.2.3. Flow-Through Diffusion Cells

Although more commonly used in permeation experiments for skin, there have been some research groups who use flow-through diffusion cells to assess

buccal permeability of compounds [128, 142–146, 153]. This technique is very similar to the Franz-type diffusion cells; however, the buccal mucosal surface is exposed to air (as there is no closed donor chamber) which can result in drying of tissue and potentially tissue death. In addition, the receptor solution flows underneath the buccal mucosa, which ensures that there is no accumulation of compound in the receptor compartment, which can often occur when using the Franz-type diffusion cells. The receptor solution, which flows underneath the tissue, collects any compound which has permeated through the tissue, and is periodically collected and analyzed to determine compound concentration.

4.5.2.4. Modified Ussing Chambers

Modified Ussing chambers have been commonly used to assess the transport of many compounds across the buccal mucosa [7, 28, 30, 41, 47, 48, 80–84, 106, 107]. The Ussing chamber, first described for investigating the transport of ions across isolated frog skin [139], has undergone a number of recent modifications, enabling convenient and rapid assessment of tissue permeability [40, 118]. This is the model which our laboratory has extensively used for assessment of buccal permeability [80–84], and it has been useful for assessing the potential of agents in enhancing permeability across the buccal mucosa. An illustration of the modified Ussing chamber commonly used in buccal mucosal permeation studies is shown in Figure 4.2.

As shown in Figure 4.2, the modified Ussing chamber system consists of two half chambers which when clamped together are separated by a piece of

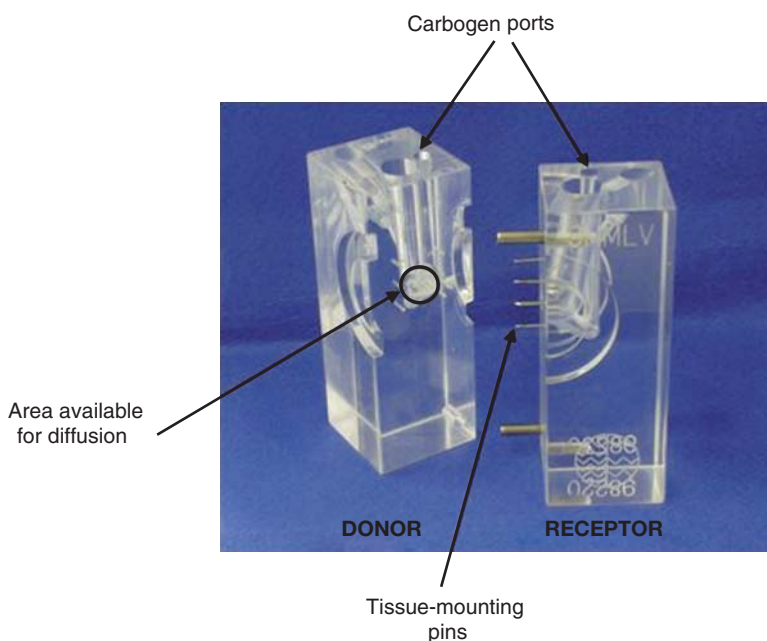


Figure 4.2 The modified Ussing chamber consisting of a donor and a receptor chamber which when clamped together are separated by a piece of tissue (intestinal, nasal, buccal, etc). The available area for diffusion (highlighted in a green circle to enhance visibility) is 0.64 cm^2 and the volume of solution applied to each chamber is 1.5 ml. The applied solution is bubbled with carbogen gas (95% O_2 + 5% CO_2) that enters the chambers through the gas ports (See also Color Insert.)

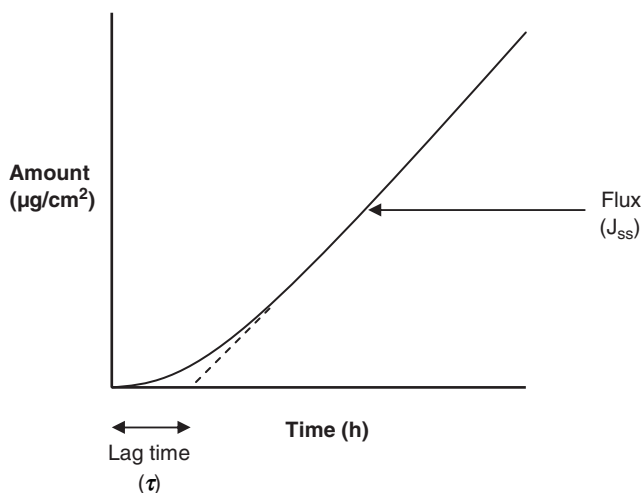


Figure 4.3 Representation of amount of drug appearing in the receptor chamber over time, where the steady-state flux (J_{ss}) is calculated from the gradient of the linear portion of the curve and the lag time (τ) is the time required for steady-state flux to be achieved.

biological tissue. A solution of drug in physiological buffer is then applied to the donor chamber and the same volume (1.5 ml) of physiological buffer is placed in the receptor chamber. The advantage of the modified Ussing chamber over the Franz-type diffusion cell and flow-through diffusion cell is that the donor and receptor chambers are provided with carbogen gas (95% O_2 + 5% CO_2), which not only provides stirring but also helps to maintain the viability of the buccal tissue [46]. The transport of compound from the donor chamber to the receptor chamber is monitored over a specified period of time, by periodic sampling of the receptor chamber. The concentration of drug appearing in the receptor chamber can then be analyzed by an appropriate detection method, and the amount of drug permeating into the receptor chamber may be plotted as a function of time, as shown in Figure 4.3.

The appropriate calculations for determining the flux and permeability coefficient across the buccal mucosa using this approach are detailed in the appendix containing the detailed method used in our laboratory for assessing buccal permeation.

4.5.2.5. Issues Associated With In Vitro Permeability Assessment

While the transport of compounds across the buccal mucosa may be assessed in a controlled environment with in vitro techniques, the ability to correlate the results obtained from the in vitro to the in vivo situation is often limited. It is thus necessary to consider the in vitro conditions and minimize, where possible, artifacts that may not be representative of the in vivo situation. This becomes particularly important when deciding on the thickness of the tissue to use in in vitro permeability experiments. When excising the buccal mucosa (as detailed in the appendix to this chapter), it is possible to use either full-thickness tissue (containing the buccal epithelium and underlying connective tissue) or buccal epithelium alone. In studies in our laboratory using both full-thickness and epithelial tissue, the presence of connective tissue significantly reduced the buccal permeation of the hydrophilic marker caffeine and the lipophilic marker estradiol [80]. However, it was observed that the difference in permeability was greater for estradiol than for caffeine, which may have been a result of the more hydrophilic nature of the connective tissue, which acts

as a greater barrier for lipophilic compounds than for hydrophilic compounds [28]. Given the blood vessels are located directly beneath the epithelial surface *in vivo*, we suggest using epithelial tissue in place of full-thickness tissue, avoiding the artificial barrier created by connective tissue in the absence of circulation.

Integrity markers are often used in *in vitro* permeability experiments to ensure that the model membrane is intact and that the observed permeability profiles of model compounds are not a result of compromised tissue integrity. One common method of assessing tissue integrity is to include a nonabsorbable marker at the completion of a permeability experiment. Our laboratory uses the high molecular weight fluorescein isothiocyanate (FITC)-labeled dextran with a molecular weight of 20 kDa as a marker of tissue integrity [80], on the basis that studies investigating the permeability of FITC-dextran have revealed that passage of such hydrophilic compounds through porcine buccal epithelium is restricted to permeants with a molecular weight less than 20 kDa [47, 48, 55]. We have demonstrated that such a high molecular weight dextran appears only in the receptor chamber following intentional tissue damage, and this was accompanied by an increase in the permeability of caffeine [80].

Because of the possible effects of active and carrier-mediated processes and metabolic biotransformation, the issue of tissue viability is important for *in vitro* buccal mucosal experiments. The barrier nature of the buccal mucosa resides in the upper layers of the epithelium, where unlike in the stratum corneum, the cells contain a variety of functional organelles [119, 122, 125, 150], and so tissue viability may be an important component of the barrier function of the tissue. Various methods have been employed to assess the viability of excised buccal mucosa, including measurement of biochemical markers, microscopic methods, and linearity of transport data [42]. While biochemical methods, including measurement of adenosine 5'-triphosphate (ATP) levels and utilization of glucose, provide information on the metabolic activity of the tissue, this does not necessarily relate to the barrier function of the tissue. In excised rabbit buccal mucosa, levels of ATP were measured and found to decline by 40% in 6 h, and this correlated well with transmission electron microscopic evaluation of the tissue (intact superficial cells) [32]. In addition, the permeability of a model peptide was unaltered up to 6 h postmortem, but at 8 h, a significant change in permeability was observed [32]. These investigators therefore claimed that excised rabbit buccal mucosa could be used for diffusion studies for ~6 h.

Recently it has been claimed that the tissue can be considered viable if the drug permeability does not change over the course of the experiment, and thus the actual permeability experiments themselves may provide insight into the viability of the tissue [109, 157]. This method was employed in permeation experiments using porcine buccal mucosa, where the permeability of compounds was assessed in two consecutive permeability experiments to ensure the nature of the barrier was not compromised [111, 112]. While this demonstrates that the barrier nature of the tissue was unaltered between the permeation experiments, the tissue may have already undergone tissue death in the time between the excision and the commencement of the initial permeation experiment, and thus the permeability rate obtained *in vitro* may not be representative of the *in vivo* situation. Therefore, more studies assessing the dependence of the barrier nature of the buccal mucosa on tissue viability are

required, especially since the role of specialized transport processes in oral mucosal permeability is becoming more appreciated.

In our laboratory, the viability of excised porcine buccal mucosa was assessed using histological evaluation and a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) biochemical assay which has previously been used in assessing the viability of excised buccal mucosa and cornea [49, 50]. Histological evaluation of tissue demonstrated that the buccal epithelium appeared viable up to 9 h postmortem, and this was supported by the MTT biochemical assay, which indicated that viability was maintained for up to 12 h [80]. Therefore, we recommend that all permeation experiments using porcine buccal mucosa be performed within 9–12 h of animal death.

While there are limitations associated with the use of an *in vitro* permeability model for assessing the transport of compounds across the buccal mucosa, it can still be useful in assessing and comparing the permeability of compounds under different conditions, such as pH, temperature, and osmolarity, which provide valuable information on the mechanisms involved in drug transport. Additionally, the preliminary effects of potential chemical penetration enhancers or formulation excipients may be assessed, and these may provide a substantial rationale for subsequently assessing the effect of these agents in man.

4.5.3. Buccal Cell Cultures

The use of buccal cell cultures for assessing the permeability of the buccal mucosa has attracted recent attention (see Chap. 7 for a more extensive summary). In order to culture buccal epithelial cells, the cells must be harvested from an appropriate source and cultured under specific conditions using an appropriate growth medium, temperature, and humidity [46]. Cell cultures have been successfully grown from hamster cheek pouch. These cultured cells, however, did not differentiate to form a complete keratinized surface as seen in the normal hamster cheek pouch, and they consequently displayed a greater permeability to compounds when compared with keratinized hamster cheek pouch mucosa [134]. Therefore, the cultured hamster cheek cells more closely mimicked the human buccal mucosa due to their lack of keratinization, and so this may be an appropriate model for predicting permeability through the human buccal mucosa.

Another cell culture model which has been proposed as a model of the human buccal epithelium is the TR146 cell line [51, 52, 85–87]. The TR146 cells originate from a human buccal carcinoma [98], and when cultured, they form an epithelium resembling that of the buccal mucosa [52], with the appropriate differentiation patterns seen in human nonkeratinized epithelium [53]. However, the TR146 cell culture model has less of a barrier nature when compared to human and porcine buccal epithelium, as demonstrated by a significantly greater permeability to tritiated water, mannitol, testosterone, dextrans, and nicotine [85, 87–89], and this may be due to the cancerous nature of the original cells.

Recently, a cell culture derived from biopsies of healthy human buccal mucosa has been developed with remarkably similar morphology, membrane-coating granule structure and appearance, and lipid composition to intact buccal tissue [102]. The barrier nature of this cell culture model is similar to intact buccal mucosa, and so this cell culture may be an alternative model to the

TR146 cell culture. With the development of tissue culture techniques, it is anticipated that various cell culture models may be developed with similar morphological and barrier properties to normal intact buccal mucosa. Such models may be very useful in assessing the buccal permeability and metabolism of many compounds.

4.6. Concluding Remarks

The buccal mucosa does serve as an alternative route for administering compounds systematically; however, to ensure particular compounds are candidates for delivery across this biological tissue, preclinical screening is essential. While in vivo human permeability studies are ideal, due to their costs and associated issues, it is necessary to perform such screening in vitro. Assessment of compound permeability across porcine buccal mucosa has been widely used and can provide the preclinical biopharmaceutical scientist with much information relating to permeability, routes of transport, and effects of various chemical penetration enhancers.

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